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Tuan A. Nguyen
Princeton University

Jasmina Brescic
Department of Chemistry and Chemical Biology

David J. Vinyard
Princeton University

Thenappan Chandrasekar
Princeton University

G. Charles Dismukes
Department of Chemistry and Chemical Biology

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Identification of an Oxygenic Reaction Center *psbADC* Operon in the Cyanobacterium *Gloeobacter violaceus* PCC 7421

Tuan A. Nguyen,†,[1] Jasmina Brescic,[2,3,4] David J. Vinyard,[1,2,4] Thenappan Chandrasekar,‡,[1] and G. Charles Dismukes*[,2,3,4]

[1]Department of Chemistry, Princeton University

[2]Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey

[3]School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey

[4]Waksman Institute of Microbiology, Piscataway, Rutgers, The State University of New Jersey

†Present address: Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA.

‡Present address: Department of Urology, University of California, Davis Medical Center.

***Corresponding author:** E-mail: dismukes@rci.rutgers.edu.

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Abstract

Gloeobacter violaceus, the earliest diverging oxyphotobacterium (cyanobacterium) on the 16S ribosomal RNA tree, has five copies of the photosystem II *psbA* gene encoding the D1 reaction center protein subunit. These copies are widely distributed throughout the 4.6 Mbp genome with only one copy colocalizing with other PSII subunits, in marked contrast to all other *psbA* genes in all publicly available sequenced genomes. A clustering of two other *psb* genes around *psbA3* (glr2322) is unique to *Gloeobacter*. We provide experimental proof for the transcription of a *psbA3DC* operon, encoding three of the five reaction center core subunits (D1, D2, and CP43). This is the first example of a transcribed gene cluster containing the D1/D2 or D1/D2/CP43 subunits of PSII in an oxygenic phototroph (prokaryotic or eukaryotic). Implications for the evolution of oxygenic photosynthesis are discussed.

Key words: *Gloeobacter violaceus*, photosystem II, type 2 reaction center.

Gloeobacter violaceus PCC 7421 is a rod-shaped unicellular cyanobacterium that, unlike all other known cyanobacteria, lacks thylakoid membranes (Rippka et al. 1974), which forces its photosynthetic machinery to operate within the cytoplasmic membrane and limits its metabolism and growth rate (Guglielmi et al. 1981). This unusual characteristic may be indicative of an early divergence in the photoautotrophs, supported by phylogenetic analysis of 16S ribosomal RNA (rRNA), placing *Gloeobacter* at the earliest branch of the cyanobacterial tree (Nelissen et al. 1995).

Cyanobacteria have one to five copies of *psbA* encoding the D1 protein of photosystem II (PSII) that are differentially expressed based on environmental conditions and have previously been described to require regulation of each *psbA* copy independent of other PSII genes (i.e., no known operon) (Mulo et al. 2009). Here, we compare the distribution of PSII genes of publically available prokaryote genomes to the synteny of reaction center genes of type 2 anoxygenic phototrophs, the postulated evolutionary precursors to oxygenic PSII. We provide evidence that a copy of *psbA* in *Gloeobacter* is cotranscribed with *psbD* and *psbC* in a single operon.

Our analysis using all available cyanobacterial genomes except *Gloeobacter*'s revealed that *psbA* genes are not cotranscribed with any other PSII-related gene. Of the 36 complete and partially assembled cyanobacterial genomes analyzed, no *psbA* gene is within 10 kbp of any other PSII gene, with the exception of another *psbA* gene (Supplementary Material online). *psbD* and *psbC*, on the other hand, are known to be organized in an operon (Holschuh et al. 1984; Bookjans et al. 1986; Golden and Steams 1988). *Gloeobacter* has five copies of *psbA*, one of which (*glr2322*, referred to in this work as *psbA3*) is physically located immediately upstream of the conserved *psbDC* operon (Nakamura et al. 2003) (fig. 1). Using the Neural Network Promoter Prediction algorithm (Reese 2001) and GeneMark Gene Prediction (Besemer and Borodovsky 2005), a strong ribosome-binding site was predicted for *psbD* in *Gloeobacter* (ACGGAG), but a promoter is not identifiable upstream of *psbD*. On the other hand, promoter elements were readily identifiable upstream of *psbD* in other cyanobacteria (e.g., promoter score of 0.95 and 0.94 for *Synechocystis* and *Prochlorococcus*, respectively). Therefore, we hypothesized that *psbDC* is cotranscribed with *psbA3* via a strong promoter identified upstream of *psbA3* (promoter score of 0.96). The intergenic distance between *psbA* and *psbD* is 131 bp (fig. 2), which is within the window allowed for an operon in cyanobacterial genomes such as *Synechocystis PCC 6803* (Moreno-Hagelsieb and Collado-Vides 2002).

Sicora et al. (2008) analyzed *psbA* expression in *Gloeobacter* under normal and stress conditions and found that *psbA3* (glr2322) makes up more than 50% of the total *psbA*

Letter

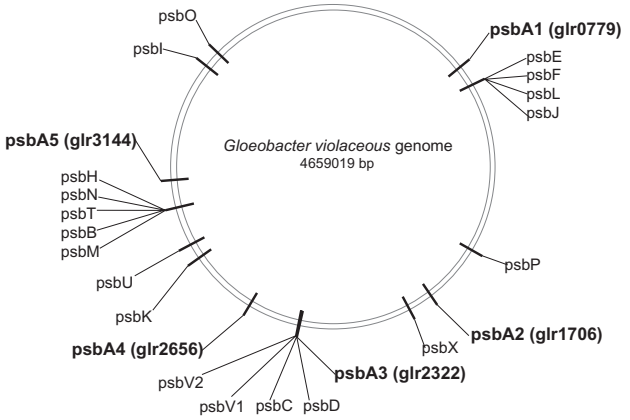

FIG. 1. Genomic map of PSII-related genes in *Gloeobacter violaceus*.

transcript pool in control, UVB, and high light conditions. This work has shown that the *psbA* gene in question here is transcribed and is also the most transcribed *psbA* gene under control and stress conditions.

Reverse transcriptase–polymerase chain reaction (RT-PCR) was employed to experimentally confirm whether the *psbA3DC* genomic region forms an operon as predicted via sequence analysis. *Gloeobacter*-culturing conditions and RT-PCR methods are detailed in Supplementary Material online. The RT-PCR results in figure 2*B* confirm the existence of a polycistronic mRNA transcript covering *psbA3*, *psbD*, and *psbC*. The long RT-PCR products (fig. 2, lane 7) confirm that the polycistronic mRNA spans all three *psbA3*, *psbD*, and *psbC* genes. These results validate the statistical prediction of a unique *psbA3DC* operon in *Gloeobacter*. To our knowledge, this is the only example of the cotranscription of *psbA* and *psbD*, encoding D1 and D2 reaction center core subunits.

We note that *psbA* and *psbD* are frequently found in bacteriophages that infect marine cyanobacteria. In these phage genomes, *psbA* and *psbD* genes are in close proximity and in the same direction due to the small phage genome size (Mann et al. 2003; Millard et al. 2004). However, the strong homology between *psbA* copies in *Gloeobacter* (≥83%) and the lack of a second *psbDC* operon decreases the likelihood that the *psbADC* operon is an artifact of a phage infection.

The origin and evolution of the oxygenic type II reaction centers are still areas of scientific debate. Theories have been proposed based on a variety of criteria from the chemistry of the water oxidation reaction (Blankenship and Hartman 1998; Dismukes et al. 2001) to the sequence and structure of the PSII subunits (Mulkidjanian and Junge 1997; Schubert et al. 1998) and other more general criteria such as 16S rRNA (Olsen et al. 1994). Based on sequence and cofactor similarity, it has been generally accepted that the four core chlorophyll-binding subunits of PSII (D1, D2, CP43, and CP47) are the result of acquisition and evolution of two RC1 antenna domains (N-terminal domain of *psaA*) and the L (*pufL*) and M (*pufM*) RC2 subunits (Schubert et al. 1998; Baymann et al. 2001; Raymond and Blankenship 2004). D1 is functionally equivalent to L (binding to the second quinone acceptor

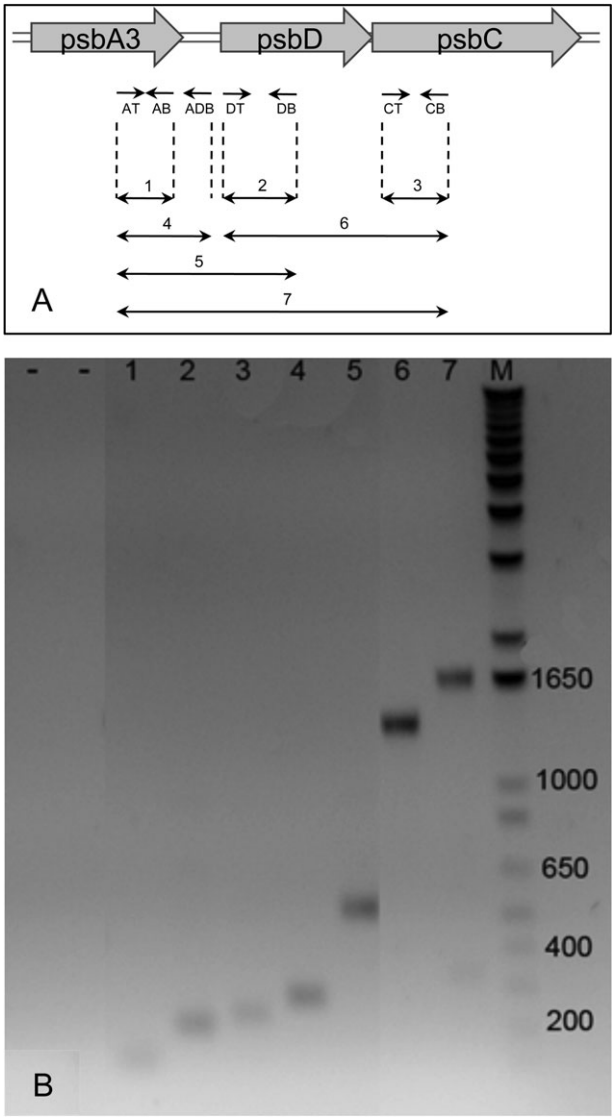

FIG. 2. (*A*) The *psbA3DC* region in *Gloeobacter*. The locations and orientations of the primers used for polymerase chain reaction (PCR) and reverse transcriptase (RT)–PCR are indicated. The primer range is indicated with lane number in figure 2*B*. (*B*) RT-PCR products provide four independent confirmations that the polycistronic mRNA covers *psbA3*, the region between *psbA3* and *psbD*, *psbD*, and *psbC*. The first two unnumbered lanes are negative controls of the RT-PCR reactions.

Q_B), and D2 is functionally equivalent to M (binding to the primary quinone acceptor Q_A) (Lockhart et al. 1996), whereas CP43 and CP47 are functionally equivalent to the N-terminal domain of homodimeric RC1 *psaA* (antenna-binding proteins with identical α-helix number and arrangement) (Schubert et al. 1998; Baymann et al. 2001).

We argue that if D1 and D2 were evolved from L and M, they should also retain a genomic arrangement similar to that of the L and M subunits in which the corresponding genes (*pufL* and *pufM*) are organized in an operon (fig. 3). This *pufLM* operon is a conserved feature of the purple and green nonsulfur bacteria (Blankenship and Hartman 1998; Sauer and Yachandra 2002). In other words, *psbA* and *psbD* may also have been arranged in an operon in the ancestral

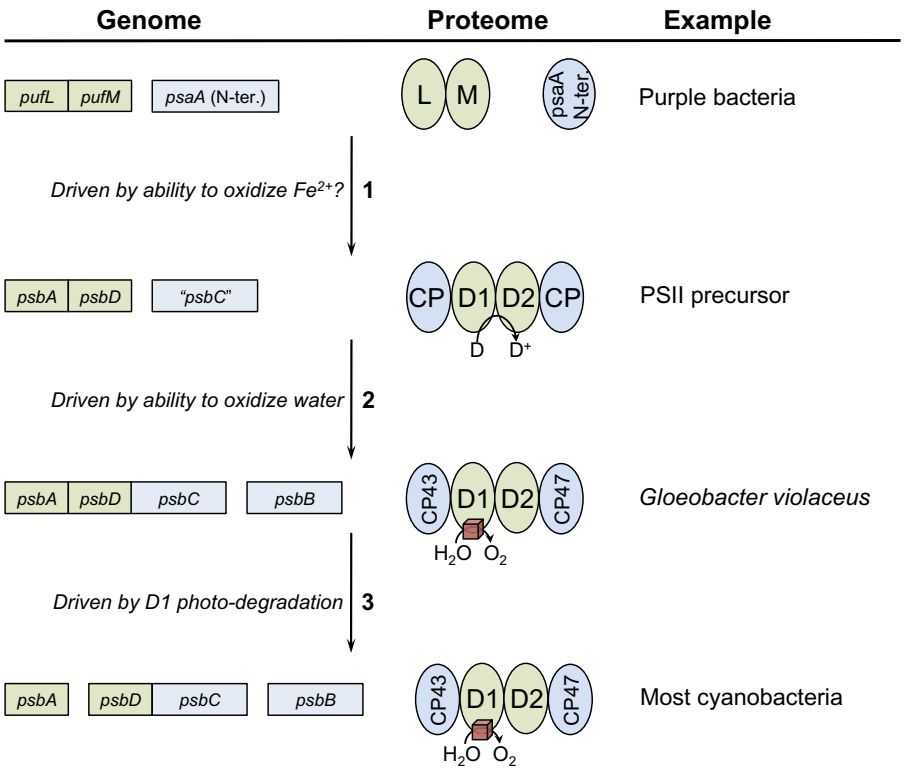

FIG. 3. A proposed model for the evolutionary significance of the *psbADC* operon. In Step 1, D1 and D2 are evolved from the L and M subunits of the RC2 of anoxygenic purple bacteria and the chlorophyll-binding protein, CP, originated from the N-terminal domain of *psaA*. Phototrophs harboring such a PSII precursor may have been capable of photochemical oxidation of ferrous or manganous minerals (e.g., Fe^{2+}/Fe^{3+} ($E_{pH\ 7} = 0.2$ V) or $Mn^{2+}(HCO_3^-)/Mn^{3+}(CO_3^{2-})$ ($E_{pH\ 7} = -0.55$ V)). In Step 2, *psbD* and *psbC* became joined in an operon—an arrangement maintained in modern oxygenic phototrophs. Also in this step, the formation of a catalytic site on D1 but not D2 led to the asymmetrical development of the two CP proteins resulting in CP43 and CP47. This protein environment enabled the formation of the Mn_4CaO_5 cluster and water oxidation. In Step 3, the need for faster turnover of D1 protein due to water oxidation damage resulted in the dissociation of the *psbADC* operon into *psbA* and *psbDC*.

precursor. Furthermore, if CP43 and CP47 are both evolved from the N-terminal domain of the homodimeric RC1 *psaA*, a common ancestor existed that we refer to herein as "CP precursor."

These arguments are the basis for an evolutionary hypothesis presented in figure 3 in which we propose that the PSII precursor was a four-subunit complex (D1, D2, and two identical "CP precursor" proteins). These four subunits were encoded by a *psbAD* operon and "*psbC*" (encoding "CP precursor") whose reaction center stoichiometry was twice that of D1 and D2. This PSII precursor may have been capable of photochemical oxidation of ferrous and manganous minerals in the Archean era but not water oxidation based on redox potential arguments. Although a PSII precursor would have had two identical CP subunits, the creation of a water-oxidizing site on D1 but not on D2 would have initiated a pressure for the differentiation of the two copies of CP, eventually becoming the CP43 and CP47 subunits found in contemporary PSII. This differentiation likely followed a gene duplication event that spatially separated the two genes in the genome. Given that D2 and CP43 would now be needed in equal quantities, *psbC* became coexpressed with *psbD* (and *psbA* in this case). *psbB* would have been individually regulated in another region of the genome.

Water oxidation chemistry causes unavoidable production of radicals that damage the surrounding D1 protein causing it to be removed and replaced much faster than all other subunits of PSII (Mulo et al. 2009). Consequently, this damage may have led to the need for faster gene turnover, which provides a selection pressure for the breakup of the *psbADC* operon. Contemporary *Gloeobacter* maintains the *psbADC* operon structure but has four other copies of *psbA* in the genome to allow independent expression of *psbA* genes relative to other PSII-related genes (Sicora et al. 2008). All other cyanobacterial genomes sequenced to date have separated this operon (*psbA* + *psbDC*) to more rapidly or efficiently repair the damaged *psbA* gene product.

In conclusion, the identification of a *psbADC* operon in *Gloeobacter*, not observed before in other oxygenic phototrophs, may have important implications in the evolution of oxygenic photosynthesis. Although the hypothesis presented here is supported by the present data, further studies are needed to confirm this model. Nevertheless, the presence of a *psbADC* operon in *Gloeobacter* is a unique characteristic among cyanobacteria.

Acknowledgments

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